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role of CCR5, however, is the regulation of immune-cell trafficking upon activation by its endogenous ligands: macrophage inflammatory protein 1α (MIP-1α), MIP-1β and RANTES (Regulated on Activation, Normal T-cell Expressed and Secreted). Since both, viral gp120 and the chemokines bind to the extracellular parts of the receptor, binding of a natural ligand, e.g. RANTES, obstructs the interaction of CCR5 with the viral protein, thereby hindering HIV infection. This makes RANTES and other chemokines potential lead structures for novel anti-HIV agents.

Here, we present advances on the study of the interactions of recombinantly-expressed CCR5 with different chemokine variants and the small-molecule inhibitor maraviroc. Experiments are performed by nuclear magnetic resonance (NMR) and surface plasmon resonance (SPR), with CCR5 incorporated in either detergent micelles, lipid bilayers, or the synthetic model membrane system of nanodiscs. Mechanistic implications of these results are discussed.

While a 3D structure of CCR5 is still lacking, these data have the potential to shed some new light on our understanding of HIV infection, as well as on the topic of cellular signalling through chemokines. As the quality of preparation of recombinant CCR5 samples improves, further milestones are expected towards structural models of the ligand-CCR5 complexes.

1211-Plat

Identification of a Small Molecule-Ligand-Binding Pocket in a G Protein-Coupled Receptor using Genetically-Encoded Photocrosslinkers

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The G protein-coupled receptor (GPCR) CC chemokine receptor 5 (CCR5) is the primary co-receptor required for HIV-1 cellular entry and the molecular target for the HIV-1 cellular entry inhibitor maraviroc. Despite the fact that maraviroc obtained FDA approval for therapeutic use in 2007, the precise mode of receptor-drug interaction and its mechanism of action has not been directly defined. Using unnatural amino acid mutagenesis and targeted photocrosslinking, we have identified amino acids in CCR5 that are within 3-5 Å of the bound ligand. We introduced *p*-benzoyl-L-phenylalanine (BzF) and *p*-azido-L-phenylalanine (AzF) at multiple specific sites in engineered CCR5 expressed in HEK-293T cells. Photocrosslinking experiments were performed in live cells in the presence of [^{3}H]maraviroc. Site-specific crosslinks were detected by scintillation counting of Western Blot membranes of cell extracts. The pattern of BzF and AzF crosslinks was compared based on existed models of CCR5. This method is an extension of earlier work in which ligand analogues with fluorescent tags were employed (1). Targeted photocrosslinking using isotopically-labelled GPCR ligands allows the direct mapping of a drug-binding site with chemical precision and can be used to discriminate among various models of receptor-drug interaction. To our knowledge, this is the first demonstration of a direct chemical crosslink between a GPCR and a native small-molecule ligand. 1. Grunbeck, *et al.* (2011) *Biochemistry* **50**, 3411-3413.

1212-Plat

Effect of Anionic Lipids on Structure and Function of Cannabinoid CB2 Receptor in Micelles and in Reconstituted Liposomes

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Human cannabinoid CB2 receptor functions in plasma membranes of cells in immune and hematopoietic systems upon binding of cannabinoid ligands. Previously we reported on functional reconstitution of the recombinant CB2 receptor into liposomes. During solubilization and purification, structure of the receptor in micelles is stabilized by adding anionic cholesteryl hemisuccinate (CHS), that is used widely to maintain the functionality of recombinant GPCR. Here we show that the functional structure of CB2 receptor in micelles can be significantly preserved by replacing CHS with phospholipids prior to reconstitution. By ligand binding studies on the reconstituted receptor performed by ^{2}H MAS NMR, that distinguishes specific binding to the receptor and non-specific binding to the lipid matrix, and by G protein activation tests, we demonstrated that the anionic 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoserine (POPS) can be almost as efficient as CHS in stabilizing the receptor in micelles while the zwitterionic PC counterpart, POPC, was much less effective. Our results obtained with various phospholipid headgroup and acyl-chain species suggest that the net negative charge as well as the specific molecular structure of the PS headgroup plays a key role in maintaining the structural integrity of the receptor in micelles. On the reconstituted CB2 receptor in liposomes, effect of negative electric potential of membrane surface on G protein activation was investigated by systematically changing the content of anionic CHS or PS. The activation was highest at an anionic lipid content of about 50 mol%. There was no correlation between the efficiency of G protein activation and an increase of hydrocarbon chain order induced by CHS or cholesterol. The results highlight importance of anionic lipids in regulating signal transduction by CB2 receptor and possibly other class-A GPCR.

1213-Plat

Dynamic Formation of a Ternary PTH Receptor-Arrestin-GS Complex: Consequences for Cell Signaling

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Arrestins and heterotrimeric G proteins (Gαβγ) regulate G-protein-coupled receptors (GPCRs) signaling and trafficking. Arrestin binding to an activated GPCR terminates receptor and G protein coupling, and promotes receptor internalization. The binding of the β-arrestins and G-proteins on agonist-bound GPCRs is thought to be mutually exclusive. Here we show that β-arrestins prolong rather that desensitize parathyroid hormone (PTH) receptor (PTHR) signaling. By using optical approaches (confocal microscopy, FRAP, TIRF, fluorescence correlation spectroscopy, and FRET) in live cells in real time we found that PTHR forms a ternary complex with β-arrestin1/2 and Gβγ subunits in response to PTH stimulation. We further confirmed the formation of a signaling PTHR−arrestin−Gβγ complex in response to PTH binding by using purified proteins in biochemical assays. Additionally, we found that the rapid ($t1/2 < 60$ s) assembly/disassembly dynamics formation of a receptor microdomain that contains PTH, PTHR, arrestin and Gβγ subunits increases the levels of G-protein activation and cAMP accumulation in magnitude and duration. These data contradicted established tenets of the regulation of GPCR signaling and raise the emerging concept that the formation of a long-lived GPCR-Gβγ-arrestin ternary complex contributes to prolonged receptor signaling by mechanisms that presumably permit multiple rounds of GαS subunit coupling and activation.

Platform: Molecular Dynamics II

1214-Plat

The Nucleotide Dependent Mechanism of Get3 as Revealed by Molecular Dynamics Simulations

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Tail-anchored (TA) proteins, which comprise approximately 5% of all transmembrane proteins, contain a single transmembrane domain (TMD) located near the C-terminus. This unique organization presents a topological problem that prevents the classical cotranslational translocation by the signal recognition particle, thus a distinct posttranslational targeting pathway is required for membrane insertion. In yeast, this is guided entry of TA-proteins (GET) pathway. At the center of this process is Get3, an enzyme that shuttles a TA protein from the cytosolic Get4/Get5/Sgt2 complex to the transmembrane Get1/Get2 site. Biochemical and structural studies have revealed that Get3 is a homodimer that leverages the power gained through ATP binding and hydrolysis to undergo large-scale conformational changes between an ''open'' and ''closed'' conformation. Through the use of conventional MD simulations, enhanced sampling, and rigorous free energy calculations in five possible symmetric and non-symmetric nucleotide states, we have mapped out the underlying potential of mean force for the protein as it progresses through the hydrolysis cycle. Results have lead to a mechanistic model that agrees well with experiments on the stabilities of the open and closed conformations in the symmetric states, and were able to not only predict the existence of the very recently discovered semi-open state, but also to closely match its structure. In addition, calculations predict the existence of a wide-open conformation, along with the probabilities of each of these states as nucleotides bind and unbind from Get3. This model improves our interpretation of experiments on Get3, suggests important details about the GET cycle, and provides a model system for understanding the coupling of nucleotide binding and hydrolysis to protein conformational changes.

1215-Plat

Simulation Study of Domains in Lipid Monolayers

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Using computer simulations we investigated the effect of coexisting phases on the properties of lipid monolayers. This is important for understanding the role of domains in surface activity of monolayers in general, and specifically in regulation of surface tension by lung surfactant. Molecular dynamics simulations with the coarse-grained force field MARTINI were employed to study